a). Amendments to the Specification

Please amend the paragraph at page 1, lines 3-5 to read as follows.

This application is a division of application No. 08/894,344 ~~filed August 15, 1997~~, now U.S. Patent No. 6,172,196.

Please amend the paragraph as page 3, lines 7-29 to read as follows.

The present invention relates to a protein having the amino acid sequence ~~represented~~ encoded by SEQ ID NO: 1, or a protein ~~being~~ capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having an amino acid sequence wherein one or more amino acid residues are added, deleted or substituted in the amino acid sequence ~~represented~~ encoded by SEQ ID NO: 1; a gene which encodes said protein; and a gene which comprises DNA having the nucleotide sequence ~~represented by~~ of SEQ ID NO: 1, or comprises DNA ~~being~~ capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having a nucleotide sequence wherein one or more ~~DNAs~~ nucleotides are added, deleted or substituted in the nucleotide sequence ~~represented by~~ of SEQ ID NO: 1. The present invention also relates to yeast belonging to the genus *Saccharomyces* and having low-temperature-sensitive fermentability which is characterized in that the above-mentioned gene on the chromosome is inactivated; dough containing said yeast; a process for making bread which comprises adding said yeast to dough; and a process for producing ethanol which comprises culturing said yeast in a medium, allowing ethanol to accumulate in the culture, and recovering ethanol from the culture.

Please amend the paragraph at page 8, lines 6-13 to read as follows.

The DNA used for the gene disruption can be prepared, for example, by a method which comprises cleavage of the gene complementing low-temperature-sensitivity with restriction enzymes to add, delete or substitute ~~DNAs,~~ nucleotides, and a method which comprises extracellular mutation (in vitro mutagenesis) of the gene complementing low-temperature-sensitivity. For the addition and substitution of ~~DNAs,~~ nucleotides, a method can be used in which the marker gene is inserted.

Please amend the paragraph at page 19, lines 4-14 to read as follows.

The above result shows that a DNA fragment which comprises the DNA fragment of about 6.5 Kbp from BamHI (A) (the sequence at positions 1291 through 1296 in the nucleotide sequence of SEQ ID NO: 1) to SphI (B) (the sequence at positions 7675 through 7680 in ~~the nucleotide sequence of~~ SEQ ID NO: 1) shown in Fig. 1 and additional sequences extending upstream of the 5' end and downstream of the 3' end of the BamHI-SphI fragment is necessary for complementing the mutation exhibiting low-temperature-sensitive fermentability of RZT-3u strain.

Please amend the paragraph starting at page 19, line 16 and ending at page 20, line 5 to read as follows.

The nucleotide sequence of the 12 Kbp DNA fragment inserted into plasmid pHK162 was determined by the dideoxy method using a DNA sequencer (Pharmacia LKB, ALF DNA Sequencer II). As a result, a gene was found which comprises the region of about

6.5 Kbp cleaved at BamHI (A) and SphI (B) shown in Fig. 1 within the open reading frame. This gene was designated CSF1 gene. As shown in ~~the amino acid sequence of~~ SEQ ID NO: 1, the polypeptide encoded by CSF1 gene which is presumed from the determined nucleotide sequence consists of 2958 amino acid residues (molecular weight: 338 kDa). DNA homology search with other genes revealed that the sequence of the upstream region in CSF1 gene comprising about 140 N-terminal amino acid residues in the open reading frame of CSF1 gene coincided with the sequence of the region located upstream of the sequence which was reported as the nucleotide sequence of GAA1 gene of *Saccharomyces cerevisiae* [Hamburger, et al.: J. Cell Biol., 129, 629-639 (1995)] (the region outside the GAA1 gene-encoding region). However, the report by Hamburger, et al. relates to GAA1 gene and contains no description about the presence of another gene (CSF1 gene) upstream from GAA1 gene. Further, in the nucleotide sequence reported by them, one base (T) is inserted between the base at position 198 (T) and the base at position 199 (G) in ~~the nucleotide sequence of~~ SEQ ID NO: 1. Thus, the polypeptide encoded by CSF1 gene ~~cannot be~~ is not anticipated ~~from the sequence reported~~ by Hamburger, et al.

Please amend the paragraph starting at page 21, line 7 and ending at page 22, line 12 to read as follows:

About 5 μg of pHK179 plasmid DNA was dissolved in 20 μl of H buffer, and 10 units each of restriction enzymes MluI and SpeI were added thereto. Reaction was carried out at 37°C for 3 hours. The reaction product was subjected to treatment for making blunt ends by using DNA Blunting Kit (Takara Shuzo Co., Ltd.), followed by separation by 0.8%

agarose gel electrophoresis. The segment of the gel containing the band of a fragment of about 10 Kbp excluding the fragment of about 0.6 kb from MluI (the sequence at positions 4388 through 4393 in ~~the nucleotide sequence of~~ SEQ ID NO: 1) to SpeI (the sequence at positions 5027 through 5032 in ~~the nucleotide sequence of~~ SEQ ID NO: 1) shown in Fig. 1 was cut out, and the fragment was extracted and purified by using GENECLEAN II Kit. Separately, about 5 μg of YEp24 plasmid DNA, which is a vector carrying the marker gene URA3 complementing uracil-requirement mutation between the HindIII sites, was dissolved in 20 μl of M buffer [10 mM Tris hydrochloride buffer (pH 7.5), 10 mM magnesium chloride, 1 mM dithiothreitol, and 50 mM sodium chloride]. Ten units of restriction enzyme HindIII was added to the solution, and reaction was carried out at 37°C for 3 hours. The reaction product was subjected to treatment for making blunt ends by using DNA Blunting Kit (Takara Shuzo Co., Ltd.), followed by separation by 0.8% agarose gel electrophoresis. The segment of the gel containing the band of a fragment of about 1.1 kb carrying URA3 was cut out, and the fragment was extracted and purified by using GENECLEAN II Kit. The DNA fragment of about 10 kb derived from plasmid pHK179 (0.5 μg) and the DNA fragment of about 1.1 kb derived from plasmid YEp24 (0.5 μg) were subjected to ligation reaction overnight at 16°C using Ligation Pack. The reaction mixture (2 μl) was used for transformation of competent high E. coli JM109 strain. The obtained transformant was smeared on LB agar medium containing 50 μg/ml ampicillin and cultured at 37°C for 20 hours. After the completion of culturing, the formed colony was isolated and cultured. A plasmid DNA was extracted and purified from the culture to obtain plasmid pHK188 for disruption of CSF1 gene. Plasmid pHK188 was confirmed to be the desired plasmid by subjecting the plasmid to 0.8% agarose

gel electrophoresis and measuring the molecular weight before and after cleavage of the plasmid with BamHI.

Please amend the Abstract at page 46 to read as follows. A clean copy of the Abstract as amended is attached as a separate sheet at Tab A.

The present invention relates to yeast having DNA encoding a protein having the amino acid sequence ~~represented by SEQ ID NO: 1,~~ of SEQ ID NO:2, or a ~~protein being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having an amino acid sequence wherein one or more amino acid residues are added, deleted or substituted in the amino acid sequence represented by SEQ ID NO: 1; a gene which encodes said protein; and a~~ gene which comprises DNA having the nucleotide sequence ~~represented by~~ of SEQ ID NO: 1~~; or comprises DNA being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having a nucleotide sequence wherein one or more DNAs are added, deleted or substituted in the nucleotide sequence represented by SEQ ID NO: 1. The present invention also relates to yeast belonging to the genus Saccharomyces and having low-temperature-sensitive fermentability which is characterized in that the above-mentioned gene on the chromosome is inactivated; dough containing said yeast; a process for making bread which comprises adding said yeast to dough; and a process for producing ethanol which comprises culturing said yeast in a medium, allowing ethanol to accumulate in the culture, and recovering ethanol from the culture~~.